observations can be linked to the reduction of the ability of EHV-4 infected PBMC to adhere to endothelial cells and therefore decrease the probability of infection due to the absence of viral transfer between EHV-4-infected PBMC and endothelial cells. We were able to show that the EHV-4 UL56 in comparison to its counterpart in EHV-1 was able to downregulate VLA-4 on equine dermal cells. Taken together, we conclude that systemic spread and higher pathogenic potential of EHV-1 compared to EHV-4 is caused by differences in their abilities to infect and/or reprogram mononuclear cells with respect to virus transfer to endothelial cells.

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Antibody and cellular immune responses of naïve mares to repeated vaccination with an inactivated equine herpesvirus vaccine

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Equine herpesvirus type 1 (EHV-1) continues to cause severe outbreaks of abortions and myeloencephalopathy in horses despite widely used vaccination. The aim of this work was to determine the effects of frequent vaccination with an inactivated EHV vaccine on immune development in horses. Fifteen EHV-1 naïve mares were vaccinated a total of 5 times over a period of 8 months with intervals of 20, 60, 90 and 60 days between vaccine administrations. Total antibody and antibody isotype responses were evaluated with a new sensitive EHV-1 Multiplex assay to glycoprotein C (gC) and gD for up to 14 months after initial vaccination. Antibodies peaked after the first two vaccine doses and then declined despite a third administration of the vaccine. The fourth vaccine dose was given at 6 months and the gC and gD antibody titers increased again. Mixed responses with increasing gC but decreasing gD antibody values were observed after the fifth vaccination at 8 months. IgG4/7 isotype responses mimicked the total Ig antibody production to vaccination most closely. Vaccination also induced short-lasting IgG1 antibodies to gC, but not to gD. EHV-1-specific cellular immunity induced by vaccination developed slower than antibodies, was dominated by IFN-γ producing T-helper 1 (Th1) cells, and was significantly increased compared to pre-vaccination values after administration of 3 vaccine doses. Decreased IFN-γ production and reduced Th1-cell induction were also observed after the second and fourth vaccination. Overall, repeated EHV vaccine administration did not always result in increasing immunity. The adverse effects on antibody and cellular immunity that were observed here when the EHV vaccine was given in short intervals might in part explain why EHV-1 outbreaks are observed despite widely used vaccination. The findings warrant further evaluation of immune responses to EHV vaccines to optimize vaccination protocols for different vaccines and horse groups at risk.

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Equine Herpesvirus-1 Interferes with Type-I IFN-Mediated Immune Responses *in vitro* in Equine Endothelial Cells

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Equine herpesvirus-1 (EHV-1) is one of the major viral pathogens of equids causing respiratory disease, abortion and neurologic disease. Recently, there have been increasing incidences of neurologic disease caused by EHV-1 in many countries. Type-I interferon (IFN) is considered to be the first line of defense against many viral pathogens. It has been reported earlier that EHV-1 infects the endothelial cells lining the blood vessels of the central nervous system and induces vasculitis leading to neurologic signs in horses. We have previously shown that the neuropathogenic T953 strain of EHV-1 suppresses type-I IFN in equine endothelial cells (EECs) (Sarkar et al., 2015). However, the antiviral effect of type-I IFN in the endothelial cells has not been studied in detail. We hypothesized that EHV-1 infection inhibits type-I IFN-mediated antiviral response. In this study we investigated the antiviral effect of type-I IFN against the neuropathogenic T953 strain of EHV-1 *in vitro* in EECs. To study the effect of type-I IFN, sub-confluent monolayers of EECs were stimulated with purified recombinant equine IFN-α (rEqIFN-α) for 24 h before infecting with T953 strain of EHV-1 or vesicular stomatitis virus expressing green fluorescent protein (VSV-GFP) at an MOI=0.1. VSV-GFP was used as a positive control for IFN-sensitive virus. At different times post infection (pi), cell supernatants were collected and titrated by plaque assay on rabbit kidney-13 cells to determine the number of virus particles. In a separate experiment, rEqIFN-α-treated EECs were resuspended into single cell suspensions and an infectious center assay was performed to determine if rEqIFN-α interfered with the infectivity of the IFN-treated cells. The results from both of the experiments demonstrated that T953 strain of EHV-1 was partially resistant to the antiviral effect of exogenously supplied rEqIFN-α. To investigate the mechanism of resistance, the effects of EHV-1 infection on JAK-STAT signaling pathways were analyzed. Confluent monolayers of EECs were infected with T953 strain of EHV-1 at an MOI of 5.0 and at different times pi (3 h and 12 h), cells were stimulated with rEqIFN-α 30 min prior to lysis for Western blotting analysis to determine the level of endogenous as well as phosphorylated STAT-1. Mock infected EECs stimulated with rEqIFN-α or MEM were used as either positive or negative controls, respectively. The results demonstrated that the virus infection not only interfered with STAT-1 phosphorylation, but also suppressed exogenous rEqIFN-α-induced STAT-1 phosphorylation. The findings were further confirmed by indirect immunofluorescence assay which showed that EHV-1 T953 strain prevented rEqIFN-α-induced nuclear translocation of STAT-1 from cytoplasm. Furthermore, this study also revealed that downstream of the JAK-STAT signaling, EHV-1 infection inhibited expression of cellular antiviral proteins including IFN-stimulated gene 56 (ISG56) and viperin. Altogether these data show that the neuropathogenic T953 strain of EHV-1 evades the host innate immune response by inhibiting the type-I IFN signaling pathway in EECs.

References:

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Changes in clinical parameters, SAA concentrations and virus-neutralising antibody titres after EHV-1 vaccination in mules and horses

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